## REMARKS

Applicant requests reconsideration of the application in view of the discussion that follows. The status of the claims as of this amendment is as follows: Claims 44-46 are pending. Claims 1-43 were previously canceled. Applicant reserves the right to file divisional applications to the separately patentable subject matter of claims 7, 8, and 10-24, and Applicant generally reserves the right to file continuation applications to the subject matter of canceled claims 1-43.

### The Amendments

The paragraph on page 9, lines 5-22, was amended to delete the language "multiple products" and insert therefor the language "detectable product." Support therefor is in the specification, for example, page 9, lines 16-18.

### Objection to the Specification

Applicant submits that the foregoing amendment to the specification obviates the objection to the specification at page 3 of the Office Action.

### Withdrawn Objection to the Specification

Applicant acknowledges the indication in the Office Action that the objection to the specification regarding the amendment to the paragraph on page 5, lines 1-14, was withdrawn.

Applicant reiterates that the context of the paragraph in which the allegedly offending sentence appears makes it clear to one of ordinary skill in the art how cleaving a linker results in release of multiple products. The language immediately preceding the above sentence states that the "method of the invention entails a first step of forming a sandwich of a first receptor bound to the sensitizer particle, an analyte or target, and a second receptor associated with multiple copies of a substrate. The substrate is attached via oxidant cleavable linker to a support or surface such as a particle to form what is referred herein as an acceptor particle. The analyte binds to the first and second receptor, drawing the catalyst and substrate in close proximity" (underlining added). Therefore, each second receptor molecule has multiple copies of

a substrate attached. When peroxide or singlet oxygen is generated, the oxidant cleavable linker is cleaved releasing multiple products, which are then detected. As can be seen, each cleavage of the cleavable linker results in release of a product and, since multiple copies of the substrate are attached, multiple products are released. As indicated in the specification, the product is distinguished from the substrate by the creation of at least one functional group in the product that was not present in the substrate (p 37, ln 10-12).

Withdrawn Rejection under 35 U.S.C. 112

Applicant acknowledges the indication in the Office Action that the rejection of claims 44-46 under the second paragraph of the above code section as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention was withdrawn.

Rejection under 35 U.S.C. 103

Claims 44-46 were rejected again under 35 U.S.C. 103(a) as being unpatentable over Bronstein, *et al.* (U.S. Patent No. 6,243,980) (Bronstein) in view of Pease, *et al.* (U.S. Patent No. 5,709,994) (Pease). The Office Action indicated that, with modification, the original grounds for rejection of claims 44-46, under 35 U.S.C. § 103(a) in view of Bronstein and Pease was maintained. The Office Action further raised new issues for Applicants' consideration in view of the teachings of Smith, *et al.,* 82 J. PHYS. CHEM. 2291 (1978) (Smith), and Holtz, *et al.*, 274 J. BIOL. CHEM. 8351 (1999) (Holtz). The teachings of Smith and Holtz, asserts the Office Action, raise new issues of inherency pertaining to the claimed "sensitizer causing formation of reactive oxygen, which cleaves the cleavable linker."

The Office Action recognizes that Bronstein does not describe "water-insoluble" solid supports. However, argues the Office Action, Pease describes "water-insoluble" solid supports (referring to the Title, "matrices") for general use in chemiluminescence assays (referring to the Abstract, fifth sentence). Thus, concludes the Office Action, it would have been obvious for a person of ordinary skill to perform the analyte determination of Bronstein with "water-insoluble" solid supports because Pease

discovered "water-insoluble" solid supports with "delayed luminescence" lifetimes, which can be modulated as a function of structure and/or composition (referring to col. 8, lines 1-9).

Applicant submits that, even if for the sake of argument one skilled in the art might combine the teachings of Bronstein and Pease, one still would not be in possession of all of the elements of the presently claimed methods. According to M.P.E.P. 2143.03, all claim limitations must be taught or suggested by the prior art in order to establish *prima facie* obviousness (citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)). "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970).

Applicant's arguments may be summarized as follows: The combined teaching of Bronstein and Pease (and further including that of Smith and Holtz), at the very least, is deficient in not disclosing or suggesting at least the following limitations of claim 44: (1) providing a reaction medium comprising the listed reactants <u>in combination</u>, (2) a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, <u>wherein the proximity of first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte</u>; (3) <u>detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in the medium</u>.

Bronstein discloses two distinct assays, namely, a heterogeneous assay and a homogeneous assay, each involving distinct reagents for each type of assay. The Office Action indicates that Bronstein's homogeneous assay embodiment is no longer referenced as is also Bronstein's discussion of photooxygenation.

In the Bronstein reference, a heterogeneous assay is described in the specification and illustrated in Figs. 2A and 2B. The Abstract of the reference discusses the heterogeneous assay in general terms. A peptide bearing a cleavage site for the protease of interest is provided with a first member of a first ligand binding pair at one end, and a first member of a second ligand binding pair at the other end. The other member of the first ligand binding pair is attached to a surface, which binds

the peptide, or protease substrate, to the surface. The peptide substrate is combined with the protease and target compound or sample. Substrate cleavage, if not inhibited, is allowed to occur, and any unbound cleaved fragments are removed. An enzyme complexed with the second member of the second ligand binding pair is added, and allowed to bind to any of the (uncleaved) first member of the second ligand binding pair remaining. Unbound complex is removed, and a 1,2-dioxetane substrate for the enzyme is added. If any peptide substrate has not been cleaved, the dioxetane will chemiluminesce, indicating inhibitory activity.

At col. 5, line 42, *et seq.*, the patentee indicates that a peptide substrate is synthesized which contains the appropriate cleavage site for the target protease. This peptide is labeled with one member of a first ligand binding pair, such as biotin, on one end and a member of a second ligand binding pair, such as fluorescein, at the other end. This peptide is then incubated, with the protease and a compound of interest to be screened for inhibitory activity, in a well or other solid phase coated with the second binding ligand of the first ligand binding pair, such as avidin or streptavidin. In this way, the biotin binds to the streptavidin and the fluorescein-labeled peptide becomes bound to the well unless the peptide was cleaved by the protease, in which case only the biotin with a peptide fragment binds to the streptavidin in the well. After incubation, the wells are washed and incubated with the second binding member of the second binding ligand pair conjugated with an enzyme, which is an effective trigger for a 1,2-dioxetane such as alkaline phosphatase. The wells are then washed and incubated with a 1,2-dioxetane substrate such as chlorine-substituted phosphate dioxetane (CSPD) (see Fig. 3) and the signal is measured. Higher signals are detected in the presence of an inhibitor. This is so because the inhibitor prevents the protease from cleaving the peptide, and the fluorescein-peptide-biotin moiety bound to the streptavidin attached to the well remains intact. Therefore, the fluorescein is available for binding to the binding member for the fluorescein (second binding member for the first binding member of the second ligand binding pair), which is conjugated to alkaline phosphatase. Thus, the alkaline phosphatase reagent remains in the well after washing and is available to act on the CSPD substrate resulting in the release of a chemiluminescent species. If the test compound is not a protease inhibitor, the

protease cleaves the peptide and there is no fluorescein-peptide-biotin moiety remaining in the well after washing.

The following scheme 1 represents the above heterogeneous assay where the compound tested for inhibition of protease activity is inhibitory (where "A" is the first member of the first ligand binding pair, "A'" is the second member of the first ligand binding pair, "B" is the first member of the second ligand binding pair, "B'" is the second member of the second ligand binding pair, "[-A'" is a surface with "A'" attached, "inhibitor(yes)" is a compound that inhibits the protease activity, "≡" represents non-covalent binding):

A-peptide-B + [-A' → [-A'≡A-peptide-B + protease + inhibitor(yes) → [-A'≡A-peptide-B →

wash → [-A'≡A-peptide-B + B'-enzyme → [-A'≡A-peptide-B≡B'-enzyme → wash →

[-A'≡A-peptide-B≡B'-enzyme + 1,2-dioxetane substrate →

[-A'≡A-peptide-B≡B'-enzyme≡1,2-dioxetane substrate → chemiluminescence

The following scheme 2 represents the above heterogeneous assay where the compound tested for inhibition of protease activity is not inhibitory (where A, A', B, B' and [-A' are as defined above and "inhibitor(no)" is a compound that does not inhibit protease activity, "peptide fragment" is a cleaved portion of the peptide):

B-peptide-A + [-A' → [-A'≡A-peptide-B + protease + inhibitor(no) →

[-A'≡A-peptide fragment + peptide fragment-B → wash →

[-A'≡A-peptide fragment + enzyme-B'→ wash →

[-A'≡A-peptide fragment + 1,2-dioxetane substrate → no chemiluminescence

As can be seen, in scheme 1, the peptide is not cleaved because the target

compound being tested is inhibitory, i.e., the target compound inhibits the protease activity of the protease and the peptide substrate is not cleaved. Thus, the enzyme complexed to the second member of the second ligand binding pair binds to the surface through the binding of the first and second members of the second ligand binding pair. When the 1,2-dioxetane substrate is added, the substrate transitorily binds to the enzyme and the 1,2-dioxetane is cleaved, thereby releasing a chemiluminescent species from the 1,2-dioxetane substrate (see Fig. 3). On the other hand, as can be seen in scheme 2, the peptide is cleaved because the target compound being tested is not inhibitory, i.e., protease activity is not inhibited. Thus, because the cleaved peptide fragment with the first member of the second ligand binding pair is removed by washing, the enzyme complexed to the second member of the second ligand binding pair does not bind to the surface through the binding of the first and second members of the second ligand binding pair. When the 1,2-dioxetane substrate is added, there is no enzyme present, to which the enzyme substrate can transitorily bind, and the 1,2-dioxetane is not cleaved. Accordingly, a chemiluminescent species is not released from the 1,2-dioxetane substrate.

The next discussion applies the designations in the Office Action to the above schemes that represent the teaching of Bronstein. Applicant does not acquiesce fully in the designations in the Office Action, but will apply them for the sake of argument. In particular, Applicant will accept, for the sake of argument only, that an oxygen anion species satisfies the present claim language of "reactive oxygen" and that the binding of an enzyme substrate to an enzyme satisfies the present claim language of "excitation of the sensitizer."

The Office Action argues, as to claim 44, that the sample corresponds to the medium, the protease inhibitor corresponds to the analyte, the adamantyl moiety corresponds to the solid support, the $OPO_3^{=}$ (presumably of the CSPD substrate) corresponds to the first specific binding pair member, the second member of the second ligand binding pair corresponds to the second specific binding pair member conjugated to an enzyme that corresponds to a sensitizer capable in its excited state of generating a reactive oxygen species, the labels for either end of the peptide are biotin and digoxin linked to the adamantyl moiety through a 1,2 dioxetane moiety,

which corresponds to digoxigenin-linked biotin linked to a support through an oxygen cleavable linker.

The following scheme 3 is based on scheme 1 above with the above designations as set forth in the Office Action:

biotin-peptide-anti-digoxin + [-avidin → [-avidin≡biotin-peptide-anti-digoxin + protease + inhibitor(yes) → [-avidin≡biotin-peptide-anti-digoxin → wash → [-avidin≡biotin-peptide-anti-digoxin + digoxin-enzyme → [-avidin≡biotin-peptide-anti-digoxin≡digoxin-enzyme → wash → [-avidin≡biotin-peptide-anti-digoxin≡digoxin-enzyme + 1,2-dioxetane substrate → [-avidin≡biotin-peptide-anti-digoxin≡digoxin-enzyme≡1,2-dioxetane substrate → chemiluminescence

As can be seen, in Bronstein's heterogeneous assay using the designations set forth in the Office Action, not all the reaction components are in a reaction medium in combination as required in claim 44. In Bronstein the protease inhibitor (analyte) is combined with protease and the biotin-peptide-anti-digoxin bound to the streptavidin on the solid surface. Unbound materials are removed, which would result in removal of the protease inhibitor (analyte), protease and any cleaved fragments of the peptide. Then, the surface with the bound biotin-peptide-anti-digoxin is mixed with the digoxin-enzyme reagent. At this point, the mixture lacks the protease inhibitor (analyte). Claim 44 requires that the medium contain all of the listed reactants in the claim ((a)-(d)) in combination.

The proposed teaching of Bronstein does not disclose or suggest a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, wherein the proximity of first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte. First, in Bronstein the protease inhibitor

(analyte) is removed in the first step of his reaction (scheme 3). Therefore, the protease inhibitor is not present to modulate the proximity of the first specific binding pair member, identified in the Office Action as $OPO_3^=$, and the second specific binding pair member, identified in the Office Action as the second ligand binding pair. (Note also that the claim calls for a second specific binding pair member, not a second binding pair. However, for the sake of argument only, Applicant will accept this designation.) The second ligand binding pair, asserts the Office Action, is bound to the enzyme of Bronstein. As can be seen from the above scheme 3 and discussion, the protease inhibitor (analyte) has already been removed prior to the introduction of the CSPD reagent having the $OPO_3^=$ moiety. Therefore, the protease inhibitor (analyte) cannot modulate the proximity of the $OPO_3^=$ and the second ligand binding pair linked to the enzyme.

The Office Action asserts that the support is the adamantyl moiety (presumably of the CSPD reagent that also contains the $OPO_3^=$ moiety). Claim 44 requires that the digoxigenin-linked biotin be linked to the support (adamantyl moiety in the designation of the Office Action) through a reactive oxygen cleavable linker (designated in the Office Action as the 1,2-dioxetane of the CSPD reagent). The only manner that the above situation can arguably be achieved is in the [-avidin≡biotin-peptide-anti-digoxin≡digoxin-enzyme≡1,2-dioxetane substrate complex of scheme 3. Assessing the above situation in a light most favorable to the assertions in the Office Action, the 1,2-dioxetane substrate is CSPD, which has a chemiluminescent moiety linked to an adamantyl moiety by means of a 1,2-dioxetane linker (chemiluminescer-1,2-dioxetane-adamantane, which, when cleaved, gives chemiluminescer plus adamantyl residue). Therefore, in the above scheme 3, the [-avidin≡biotin-peptide-anti-digoxin≡digoxin-enzyme≡1,2-dioxetane substrate, upon cleavage of the 1,2-dioxetane becomes [-avidin≡biotin-peptide-anti-digoxin≡digoxin-enzyme plus free chemiluminescer. The chemiluminescence from the chemiluminescent moiety (chemiluminescer) of CSPD is detected. There is no detection of released digoxigenin-linked biotin and there is no relation between the amount of released digoxigenin-linked biotin to the amount of protease inhibitor in the medium. First, there is no protease inhibitor (analyte) in the medium at this point. Second, only chemiluminescence is detected in Bronstein. Third,

the digoxin-peptide-biotin species in Bronstein is still linked to the surface by means of the binding between the biotin of the peptide and the avidin of the surface.

The Office Action asserts that the claim language "detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in said medium" is satisfied by the last sentence of the Abstract, which states "when the dioxetane is caused to decompose, energy is transferred to the fluorescing entity, which releases light of a wavelength recognizably distinct." This assertion is not convincing. First, the disclosure in the Abstract to which the Office Action refers relates to the homogeneous assay of Bronstein, not the heterogeneous assay of Bronstein. The Office Action indicated on page 8 that the revised rejection no longer references the homogeneous assay embodiment. More importantly, in the embodiment imagined in the Office Action having digoxigenin-linked biotin, there is no longer a fluorescent moiety linked to the peptide since the Office Action argued in the rejection that the fluorescent moiety is replaced by digoxin. Therefore, there would not be any fluorescent moiety to which the energy from the decomposed dioxetane could be transferred. In order for the CSPD reagent of Bronstein to work, the peptide would have to include fluorescein or a fluorescent moiety for the energy transfer to occur. Thus, substituting digoxin or digoxigenin for fluorescein would render the method of Bronstein inoperable.

Even where biotin is employed as the first member of the first ligand binding pair linked to a fluorescein-labeled peptide substrate as disclosed in Bronstein at col. 5, lines 43-48, this reagent and its use in the protease inhibitor assay of the reference does not satisfy the claim language of claim 44. The claim recites digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker. In the heterogeneous assay of the reference, cleavage of the peptide, when it occurs, releases a peptide fragment with fluorescein attached but the biotin moiety is left bound to the streptavidin attached to the well. Accordingly, even if for the sake of argument, digoxigenin were used in place of fluorescein (a proposition that is unsupported by any teaching in the reference and further it is unclear how Bronstein's method would work without the fluorescein moiety), cleavage would release a peptide fragment with only digoxigenin attached and not biotin. Accordingly, a digoxigenin-

linked biotin would not be released from a solid support by a cleavage reaction and, furthermore, a digoxigenin-linked biotin would not be detected, all of which is required by the claim language.

Although Applicant has not applied the designations in the Office Action to scheme 2 above where the target compound is not a protease inhibitor, it is readily apparent that such an application is even further removed from the presently claimed methods. When the target compound is not inhibitory, the anti-digoxin is cleaved from the solid support (well) and removed by washing, along with the target compound, very early in the reaction scheme. Thus, when a digoxin-enzyme reagent is added, there is no anti-digoxin to which the digoxin can bind and the digoxin-enzyme reagent is then removed in a wash step. It is clear that in such a scenario at least the above claim limitations are not met or suggested.

The method of claim 44 is a homogeneous assay method wherein all of the reactants are combined and the combination (without separation or removal) is incubated. Then, the combination is subjected to excitation and examined for detecting released digoxigenin-linked biotin. On the other hand, the method of Bronstein is heterogeneous, which means that one or more separation (removal) steps are carried out between addition of various reactants. As mentioned above, this is set forth in Bronstein in, among others, the Abstract.

The Office Action asserts that one skilled in the art would recognize that the adamantyl moiety of the CSPD reagent of Bronstein could be a water insoluble solid support. It would have been obvious for a person of ordinary skill, contends the Office, to perform the analyte determination of Bronstein using "water-insoluble" solid supports because Pease discovered "water-insoluble" solid supports with "delayed luminescence" lifetimes, which can be modulated as a function of structure and/or composition (referring to col. 8, lines 1-9).

As can be seen from Fig. 3 of Bronstein, cleavage of the 1,2-dioxetane results in release of a chemiluminescent moiety, which is detected. Substituting a water insoluble support for the adamantyl moiety provides no apparent advantages to this reaction and detection. The advantages recited by the Office Action appear to be irrelevant to the chemistry of Bronstein, whose method of Fig. 3 relates to a

heterogeneous assay that already utilizes a solid phase (coated plate, see Figs 2A and 2B). The skilled artisan would not envision any need or benefit from using a second solid phase (coated plate and a water insoluble support to which the chemiluminescent moiety is attached). The CSPD reagent is added to the plate after the protease inhibition reaction has been allowed to proceed and the plate has been washed. Having the CSPD reagent in a solid phase would arguably reduce the efficiency of diffusion of the CSPD reagent and reduce the ability of the enzyme to interact with its substrate in Bronstein's method.

The Office Action has attempted to piece together portions of the above distinct assays of Bronstein in an effort to produce the presently claimed methods. As discussed above, even the pieced together portions are deficient in not teaching or suggesting the elements of the present claims. Furthermore, the Office Action is required to consider all that a reference discloses; piecemeal reconstruction of the prior art is not allowed. It is not permissible to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. *In re Wesslan*, 147 USPQ 391, 827 O.G. 348 (1966).

Claim 45 is patentable over the combined teachings of Bronstein and Pease at least by virtue of its dependency from claim 44, which is patentable over the combined teachings of the references as discussed above. If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) (M.P.E.P. 2143.03).

Claim 46 depends from claim 44 and includes the additional limitation that the step of detecting the released digoxigenin-linked biotin is carried out by a detection method employing, as a third specific binding pair member, avidin bound to a member of a signal producing system or anti-digoxigenin antibodies bound to a member of a signal producing system or both. The combined teachings of Bronstein and Pease do not teach this limitation of claim 46. Furthermore, there is nothing on the record indicating where the combination of the teachings of Bronstein and Pease teach or suggest such a limitation. Therefore, claim 46 is patentable over Bronstein and Pease,

which in combination fail to disclose or suggest the above limitation as well as the limitations of the base claim, namely, claim 44, as enumerated above.

Conclusion

Applicant has demonstrated that claims 44-46 satisfy the requirements of 35 U.S.C. 103. Furthermore, the amendment to the specification avoids the objection to the specification. Allowance of the above-identified patent application, it is submitted, is in order.

Respectfully submitted,

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